

We claim:

1. A method for preparing a population of cRNA which represents transcriptional expression of cells in a sample, comprising the steps of:

reverse transcribing a population of mRNA derived from cells in a sample to form a population of first strand cDNA hybridized to said mRNA, wherein said reverse transcribing employs a primer having a first portion which is complementary to a plurality of mRNA molecules in said population, and a second portion which is a promoter sequence, wherein the first portion is 3' to said second portion and the promoter sequence is in an antisense orientation with respect to the mRNA;

denaturing hybrids of said first strand cDNA and said mRNA;

hybridizing a population of random oligomers to said population of first strand cDNA;

synthesizing second strand cDNA complementary to said population of first strand cDNA by extending said random oligomers, to form a population of double stranded cDNA each having a first and a second strand of cDNA, wherein said first strand of cDNA comprises said promoter sequence at its 5' end;

transcribing double stranded cDNA of said population using an RNA polymerase to form a population of cRNA which is antisense with respect to mRNA in said population of mRNA, wherein said population of cRNA represents transcriptional expression of cells in the sample.

2. The method of claim 1 further comprising the step of:

quantitating one or more cRNA species within the population of cRNA to assess expression of the one or more cRNA species within cells of the sample.

3. The method of claim 2 wherein a plurality of cRNA species are quantitated.
4. The method of claim 1 wherein the promoter is phage T3 promoter.
5. The method of claim 1 wherein the promoter is phage SP6 promoter.

6. The method of claim 1 wherein the promoter is phage T7 promoter.
7. The method of claim 1 wherein the random oligomers are of a uniform length.
8. The method of claim 1 wherein the random oligomers are hexamers.
9. The method of claim 1 wherein the random oligomers are between 6 and 15 nucleotides in length.
10. The method of claim 1 wherein the population of random oligomers is added at a weight ratio of between 1:100 to 10:1 of oligomer to mRNA.
11. The method of claim 1 wherein the second strand cDNA is synthesized using Klenow fragment of DNA polymerase I.
12. The method of claim 1 wherein the second strand cDNA is synthesized using T4 DNA polymerase.
13. The method of claim 1 wherein the second strand cDNA is synthesized using *E. coli* DNA polymerase I alone or in conjunction with a DNA ligase.
14. The method of claim 1 wherein the step of denaturing is performed by heating to a temperature at which less than 50% of the hybrids remain annealed.
15. The method of claim 1 wherein the first portion of said primers comprises a poly deoxythymidylate (poly dT) sequence.
16. The method of claim 2 wherein the step of quantitating employs hybridization to an array of oligonucleotide probes.
17. The method of claim 1 wherein the step of transcribing is a linear process.
18. A method for preparing a population of cRNA which represents transcriptional expression of cells in a sample, comprising the steps of:

reverse transcribing a population of mRNA derived from cells in a sample to form a population of first strand cDNA hybridized to said mRNA, wherein said reverse transcribing employs a primer having a first portion which is *polydeoxythymidylate*, and a second portion which is a phage promoter sequence, wherein the first portion is 3' to said second

portion and the promoter sequence is in an antisense orientation with respect to the mRNA;

heat denaturing hybrids of said first strand cDNA and said mRNA;

hybridizing a population of random oligomer primers to said first strand cDNA population;

synthesizing second strand cDNA by extending said population of random oligomer primers, to form a population of double stranded cDNA each having a first and a second strand of cDNA, wherein said first strand of cDNA comprises said promoter at its 5' end;

transcribing double stranded cDNA of said population using an RNA polymerase to form a population of cRNA which is antisense with respect to mRNA in said population of mRNA, wherein said population of cRNA represents transcriptional expression of cells in the sample.

19. The method of claim 18 wherein a plurality of cRNA species are quantitated.
19. The method of claim 18 wherein the promoter is phage T3 promoter.
20. The method of claim 18 wherein the promoter is phage SP6 promoter.
21. The method of claim 18 wherein the promoter is phage T7 promoter.
22. The method of claim 18 wherein the random oligomers are of a uniform length.
23. The method of claim 18 wherein the random oligomers are hexamers.
24. The method of claim 18 wherein the random oligomers are between 6 and 15 nucleotides in length.
25. The method of claim 18 wherein the population of random oligomers is added at a weight ratio of between 1:100 to 10:1 of oligomer to mRNA.
26. The method of claim 18 wherein the second strand cDNA is synthesized using Klenow fragment of DNA polymerase I.
27. The method of claim 18 wherein the second strand cDNA is synthesized using T4 DNA polymerase.
28. The method of claim 18 wherein the second strand cDNA is synthesized using *E. coli* polymerase I alone or in conjunction with a DNA ligase.

29. The method of claim 18 wherein the step of denaturing is performed by heating to a temperature at which less than 50% of the hybrids remain annealed.
30. The method of claim 19 wherein the step of quantitating employs hybridization to an array of oligonucleotide probes.
31. The method of claim 18 wherein the step of transcribing is a linear process.
32. A method for determining expression of a plurality of mRNA species in a biological sample, comprising the steps of:

reverse transcribing a population of mRNA derived from cells in a sample to form a population of first strand cDNA hybridized to said mRNA, wherein said reverse transcribing employs a primer having a first portion which is *polydeoxythymidylate*, and a second portion which is a phage promoter sequence, wherein the first portion is 3' to said second portion and the promoter sequence is in an antisense orientation with respect to the mRNA;

heat denaturing hybrids of said first strand cDNA and said mRNA;

hybridizing a population of random hexamer primers to said first strand cDNA population;

synthesizing second strand cDNA by extending said population of random hexamer primers, to form a population of double stranded cDNA each having a first and a second strand of cDNA, wherein said first strand of cDNA comprises said promoter at its 5' end;

transcribing double stranded cDNA of said population using an RNA polymerase to form a population of cRNA which is antisense with respect to mRNA in said population of mRNA, wherein said population of cRNA represents transcriptional expression of cells in the sample;

quantitating a plurality of cRNA species in the population by hybridization to an array of oligonucleotide probes.

33. The method of claim 1 wherein the step of transcribing double stranded cDNA employs labeled ribonucleotides.
34. The method of claim 33 wherein the labeled ribonucleotides are biotinylated.
35. The method of claim 33 wherein the labeled ribonucleotides are fluorescently labeled.

36. The method of claim 18 wherein the step of transcribing double stranded cDNA employs labeled ribonucleotides.
37. The method of claim 36 wherein the labeled ribonucleotides are biotinylated.
38. The method of claim 36 wherein the labeled ribonucleotides are fluorescently labeled.
39. The method of claim 32 wherein the step of transcribing double stranded cDNA employs labeled ribonucleotides.
40. The method of claim 39 wherein the labeled ribonucleotides are biotinylated.
41. The method of claim 39 wherein the labeled ribonucleotides are fluorescently labeled.
42. The method of claim 1 wherein the first portion of said primers comprises random oligomers.